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THE CHROMOSOME COMPLEX OF SYRBULA ADMIRABILIS, *W. R. B. Robertson.*

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THE CHROMOSOME COMPLEX OF SYRBULA ADMIRABILIS.

BY W. R. B. ROBERTSON.

(Contribution from the Zoölogical Laboratory, No. 180.)

Submitted in partial fulfillment of the requirements for the degree of Master of Arts.

Plates XVIII to XXII.

THIS paper is the first of a series by the author, and one of a larger series from this department, in which will be recorded the attempts to find the relation existing between body characters and germ-cell structure. Much detail has been necessary in this preliminary work in order to lay a foundation for future comparisons between the four species of *Syrbula*, and also to clear up the misunderstandings concerning the phenomena of maturation in this genus that have been brought about by Montgomery ('05) in his investigations upon *Syrbula acuticornis*. This latter purpose is of particular importance since Montgomery's findings form the only recorded exception to the rule of uniformity in the entire Acrididæan family.

Syrbula admirabilis belongs to the short-horned grasshopper family *Acrididæ* and to the sub-family *Truxalinæ*. It is found most commonly in the central part of the United States. The three other species, *acuticornis*, *fusca-vittata*, and *montezuma*, are southern in their habitat, occurring chiefly in the southwestern states and northern Mexico.

The material for these observations was obtained in Dickinson county, Kansas, during July, 1906. Flemming's fluid was used for fixation. Heidenhain's iron-hæmatoxylin was found best for staining during the metaphase of mitosis and Flemming's tricolor the most desirable for all other stages, especially where it was desired to distinguish between the accessory chromosome and the structures commonly called nu-

cleoli. The ribbons were cut usually twelve micra thick, in order to make it possible for a whole nucleus to be seen in one section, thus avoiding the uncertainties arising in the counting of chromosomes, etc., from the use of fragmentary nuclei.

METAPHASE OF THE SPERMATOGONIA.

For convenience, the chromosomes have been numbered according to their size, from smallest to largest, the system being based upon the relative sizes of the twelve chromosomes in the first spermatocyte. In polar views of the spermatogonial metaphase twenty-three chromosomes can be very clearly seen, and it is apparent, as Sutton and Montgomery have pointed out, that they occur in pairs. They have therefore been numbered in duplicate according to similarity of size. In doing this it is assumed that the largest two of the twenty-three, the No. 12's, will conjugate and form the largest, No. 12, of the twelve first spermatocyte chromosomes; that the next largest two, No. 11's, of the spermatogonia will form the one next largest, No. 11, of the spermatocyte; and so on for each pair. In this way the twenty-three chromosomes of a spermatogonium may be grouped into a series of eleven pairs and one odd unpaired member. The odd one is probably the assessory chromosome, although we cannot be absolutely sure here which one of the complex it is. This much, however, can be said; there are three chromosomes, the two No. 10's and No. 5 (figs. 1, 2, 4 and 6), which are of about the same size. In synapsis two of these will unite, and, of course, one of them must be left unpaired (Wilson, '05). This, then, is thought to be the accessory. It corresponds in size with the accessory of the first spermatocyte, where there can be no doubt as to its identity.

Six polar views were drawn, four of which, figures 1-4, are from one animal and one apiece, figures 5 and 6, from two other animals. Of the chromosomes in each cell there can be distinguished three large pairs, the 12's, 11's and 10's (Montgomery, '05); an accessory No. 5, which, as before said, is about the size of the No. 10's; three pairs of very small ones, and five pairs ranging in size between the largest and the smallest. Usually the No. 4's and 6's may be recognized, but the 7's, 8's and 9's, being very much alike in size, can hardly be told apart. In the drawings it is not possible in every case to show the real length or size of the chromosomes, since they

do not always lie in a plane parallel with the field of the objective. The image is thus foreshortened. The proper size relations, however, are quite apparent to the eye of the observer as he examines the chromosomes by different focusings.

In mitosis every chromosome divides longitudinally. The splitting seems to have already taken place when the elements first appear in the equatorial plate. (Figs. 1-4.) The separation of the halves begins at that end of the chromosome which is turned towards the center of the plate (fig. 6), the end to which the fibers from each pole of the spindle are attached. It seems sometimes that the halves are moving apart at the outer end of the chromosome but this only indicates that the element is already split. The real separation of the halves begins at the inner end.

At this point it may be well to call attention to the position of the members of a pair in the plate. The two *may* lie together but it does not follow that they always do so. In the case of the smaller chromosomes the members of a pair are quite often on entirely opposite sides of the plate, or on the same side but with several other chromosomes between them. Compare figure 6, chromosomes 4, 4 and 3, 3; and figure 3, chromosomes 3, 3 and 2, 2. In figure 6, No. 4 on the right side has no member near it that at all corresponds in size, and yet we know that it will unite only with one which is its equal. We know this from the fact that the halves of a tetrad are always alike in size, length and diameter. The only chromosomes with which this one can at all conjugate are on the opposite side of the plate.

THE ACCESSORY CHROMOSOME AND THE NUCLEOLI.

In studying the resting stages of the spermatogonial and spermatocyte nuclei the chief object has been to trace the accessory, to see if there is any connection between it and the nucleolar-like structures that are always present in these stages of the nucleus. The earliest of the secondary spermatogonial nuclei that were found (figs. 7, 8 and 9) showed the presence of these nucleolar structures. With Flemming's tricolor they take safranin, like chromosomes in the condensed or homogeneous condition (figs. 7-10). Distinct from the nucleoli the accessory chromosome lies at one side of the nucleus, separated from the rest of the chromosomes in a sac or vesicle of its own (figs. 7-11). It shows no evidence of being

a paired structure as Montgomery ('05) suggested. In late anaphases and early prophases of the spermatogonia the other chromosomes also seem each to be enclosed, more or less, in separate sacs or pouches, which are, however, in communication with each other by means of the larger cavity of the nucleus into which they all open. These sacculations are, however, not nearly so prominent as that of the accessory. These observations agree with the conditions that Sutton ('00) found in *Brachystola magna*. In the earlier cells the accessory shows that it is reticular in structure, like other chromosomes, though not inclined to open out so much as they. The nucleoli in these cells (fig. 7) do not stain so intensely as they do afterwards.

Figures 8 and 9 represent a somewhat later stage, possibly the telophase between the last two spermatogonial divisions. The reticular structure of the accessory still persists. It is much like the other chromosomes, but just a little more regular in outline and more condensed. The membrane or sac enclosing the accessory in figure 8 seems to extend into the center of the nucleus, but this is only apparent. Instead it lies in the upper part, extending over the inner side of the upper wall. Figure 9 is a cell from the same cyst, showing a cross-section of the accessory in its sac. In focusing through the cell from top to bottom it can be seen without doubt that this is the same structure found in the preceding cell. The other chromosomes in this cell appear also in cross-section. Two nucleoli seem to be present in every cell of the cyst. They are somewhat larger than in figure 7 and stain more intensely.

After the last spermatogonial division the nucleus is at its smallest volume. The chromosomes, except the accessory, loosen up and take on a reticular condition. The accessory retains its homogeneous and more or less condensed state, staining red with the tricolor (fig. 10). It becomes very irregular in outline, thus indicating a tendency to behave like the other chromosomes. The nucleoli are present and stain red, though not so intensely as does the accessory. Figure 11 is a still later stage, where the chromatin has become very much loosened. The nucleoli seem to share this same condition of loosening, as they are stained very pale, almost a violet, but instead of becoming granular they continue to be homogeneous.

From figure 12 on, the nuclei belong to the spermato-

cyte stages, as shown by their increased size. The chromatin of the ordinary chromosomes seems to be strung out in granules along the linin threads, giving a somewhat bead-like appearance. The accessory, instead of being elongated and stretched upon the nuclear wall as before, seems now to be massed into a sort of irregular lump, which, however, still clings to the wall. The nucleoli here stain deeply with the safranin. In each of the four cells (figs. 12-15), all of which are from the same cyst, two nucleoli seem still to be present. Sometimes they lie very close together, even in contact (figs. 14 and 15). In addition there is in some cells, but not in every one, another body (*K*, figs. 11, 12, 13) somewhat similar to the others but more irregular and diffuse than they. It may possibly be a portion of a chromosome that has not entirely disintegrated or loosened up since the last mitosis, or it may be a spermatocyte chromosome condensing ahead of its fellows. At the proximal end of this follicle, where the cells are older and farther advanced, and from which figures 10-15 were taken, there is present only a single nucleolar structure, besides the accessory, in each nucleus. The nuclei here, however, were not very well stained, so figures 16-22 were taken from the proximal part of another follicle of the same testis. They show the same stages as the preceding follicle, but much better stained.

In the spermatogonia, and in the earlier stages of the growth period, as I have before indicated, there appear to be two nucleolar bodies present. In the later growth stages (figs. 16-26) there is only one that is at all a permanent structure. This is about equal in size to two of the former nucleoli. (Compare figures 14, 15 and 18.) From these observations it seems possible that there may be a conjugation of these structures in the earlier stages to form the single structure of the later stages. But the proof of the point will have to be established by further investigation. The single nucleolar body, as it occurs in the later stage, may be distinguished from the accessory in three ways: (*a*) by its smaller size; (*b*) by the fact that it usually lies nearer the center of the nucleus, not against the nuclear wall, as does the accessory; and (*c*) because in the later stages it gradually loses its ability to hold the safranin stain (figs. 20-26). The time at which the body begins to lose its staining ability seems to

vary. Possibly this is due to variations in the extraction of the stain during the clearing process. The nucleolus in figures 16-19 stains as deeply as does the accessory. In some cysts, where the chromosomes are in the extremely loose or early prophase stage, it may be stained very lightly (figs. 20-22)—a violet instead of a safranin color—while in other cysts, where the chromosomes are in a much more advanced condition, it may still be found staining as homogeneously and as deeply as the accessory, though much more irregular in outline than the latter. However, by the time that the chromosomes begin to condense it has almost entirely lost its color, as in figure 24. Finally, at the stage of figure 26, we find it nothing but a pale-yellow or colorless mass. Along with the loss of staining capacity comes a slight decrease in volume. But although it may appear irregular in outline and colorless, it always retains its homogeneous structure. A count of the chromosomes at the stage of figure 26 was made, which showed eleven ordinary chromosomes, the accessory and one nucleolus. For this reason, as well as others, we are quite safe in contending that this permanent structure is not a chromosome but a nucleolus, and we feel still more certain that it is not a second accessory or heterochromosome as Montgomery has described it for *Syrbula.*

In figure 19 is represented a second densely staining body (*t*). This seems to be merely the end of a spermatocyte chromosome, as indicated by the figure, that is beginning to condense ahead of its fellows. It cannot be typical of the cyst, for no other cells were found in the cyst containing an element that could be considered homologous to this. Possibly it is the same structure as seen in *K*, figures 11, 12 and 13. There are present at times small globules, such as *P*, figures 16 and 18. They are by no means constant for every cell and therefore have been disregarded.

Turning to the accessory again, we find it changing from the smooth, compact, lumpy condition of figures 16-19 to a flat, extended condition as in figures 20-22. Sometimes in this state it is extremely ragged (fig. 20) and may be vacuolated (fig. 21), as is often the case in the earlier (lump) condition (fig. 18). In the stage following (figs. 23-28), the accessory as usual manifests its tendency to behave like other chromosomes, by evolving out of the flat, somewhat fenestrated mass

of such as figure 20 a coiled spireme similar to what McClung ('02) has described for the *Locustidæ*. At first this spireme is very irregular and difficult to outline (fig. 23), but later it becomes as clear cut as any ordinary metaphase chromosome, though much more coiled (figs. 24, 25). It gradually shortens and thickens, as shown in 26, 27 and 28, and finally arrives at the metaphase as a short, compact, almost straight rod (figs. 30-36). The accessories of figure 25 are from nuclei of the cyst which contains figure 24, those of figure 27 from that containing figure 26, and those of figure 28 from the cells of a cyst that is still farther advanced than any of these. As well as showing the gradual shortening and thickening that the accessory undergoes, these figures show the many shapes that it may assume in various nuclei that are at the same stage in development. From the time that it enters the spireme condition it becomes perfectly smooth in contour, contrary to what we find in many *Acrididæ*, where in the late prophase or the metaphase it is often woolly. No indication of a longitudinal split has been noticed in this spireme condition, although it might be expected to be present. And lastly, there is no evidence of a transverse constriction such as Montgomery claims for *S. acuticornis*.

We have now traced the accessory from the resting period of the spermatogonia through to the spermatocytes. At times there was difficulty in recognizing it, especially in the spermatogonial prophase, where it had a tendency to form a network like the other chromosomes, but even there we were able to tell it by its peculiar vesicle. After the last spermatogonial division it was always recognizable by its condensed, homogeneous appearance and by its peculiar position, applied close against the inner side of the nuclear membrane. During all this time there has been no indication of its being a paired or bivalent structure. We have seen no segmentation of the spireme in the vesicle stage of the spermatogonia, no paired condition in the spermatogonial metaphase where the chromosome number was odd, not even, and no paired condition in the growth period following the last spermatogonial division, where the additional chromatin-like structure present was found to be a nucleolus, not a chromosome. And lastly, we have seen no transverse constriction or a bending at any particular place that would in any way indicate the bivalency

of this element. To the contrary, we have found it always and under all conditions an independent, odd, unpaired and univalent member of the chromosome complex.

THE CHROMOSOMES IN THE SPERMATOCYTES.

It is at this stage in the course of the development of the germ-cells that the characteristics of the various chromosomes of the complex stand out most prominently. The drawings were made principally from polar views of the cell-plate. A few lateral views were drawn to show in particular the habit of the accessory. To understand the shapes assumed by the various chromosomes during the mitotic stages attention should be called to the forms they assume in the prophase just before the formation of the mitotic figure. Tetrad formation has already received such a thorough discussion by McClung ('00 and '02) that it seems unnecessary to say anything further about it, but the mistakes of some recent workers along this line make it again necessary to bring up the subject. In figures 26 and 29 are shown the different forms of rods, rings, crosses, V's, Y's, etc., that may be seen in the late prophase stages. As a study of the figures will show, "all of these shapes may be reduced to a single type, that of a rod with one longitudinal and one cross division." Every rod is formed by the end-to-end union of two homologous spermatogonial chromosomes. In this union like ends always unite with like ends, *i. e.*, proximal with proximal and distal with distal, if the latter unite. By "proximal" is meant that end of the chromosome which is always turned towards the center of the cell-plate and to which the spindle fibers are attached, whether in spermatogonia or spermatocytes (figs. 1-6 and 30-36). By "distal" of course is meant the opposite or outer end. In this conjugation of the spermatogonial pairs to form the tetrads the proximal ends always unite, but the distal may or may not. If they do there are formed rings, figure 8's, etc. If they bend toward each other but do not unite there occur partially closed rings, V's, bent rods, or kidney shapes. If the members of the pair lie fully extended there is produced simply a straight rod.

At the point where the members of the pair always unite, the proximal end, the chromatids (halves of the spermatogonial chromosomes) may quite often have started to move outward in the plane of the cross-division (*XX* in fig. 29).

This usually gives an enlargement at this point, or, if far enough along, a transverse extension. The point at which the distal ends unite, when they do so in forming a ring, is often marked by a constriction. The signs *XX* mark the points at which the spindle fibers will be attached, and also where the future cross or reduction division in the second spermatocytes will take place. The degree to which the chromatids have moved along the transverse axis determines the shape of the tetrad. In this way we account for the various crosses that are found. If the chromatids have moved out very far, the long axis of the cross may be in the transverse direction. This condition may be seen in the long drawn-out chromosomes of the lateral views (figs. 34 and 35). Exactly the same phenomena may occur in the case of the ring forms. The only difference with these is that the ends of the longitudinal or primary axis of the cross are turned over toward each other and united end to end, thus forming a ring. As the arms of the transverse axis elongate or pull out, the ring (arms of the longitudinal axis) keeps getting smaller (fig. 29) until there is produced the same result as with the cross forms. One of these rings acting in this manner is seen in figure 29. From the figures in 29 we are led to believe that the greater part of this gliding of the chromatids from the longitudinal to the transverse axis is done while the chromosomes are still in the prophase condition, before they enter the metaphase and have had a chance to come under the influence of the spindle fibers. This seems to indicate that chromosomes are to a certain extent automatic in the division process. Lastly, a word should be said in regard to the puzzling appearance of some of the strictly cross forms. The angles that the limbs of the cross may have with each other, depending upon the position of the tetrad in the cell, often give to such chromosomes the appearance of K's, Y's, etc., but with careful observation it may easily be seen that they are of the cross type. The figures themselves (fig. 29) furnish a better explanation of this than is possible by the use of words.

It is from prophase chromosomes such as we have been describing that the metaphase chromosomes of the first spermatocyte have been derived. The only difference is that the latter are much more condensed, even so much so that the longitudinal split is no longer visible. But the same four

parts are present and in the same relation to each other. Of course now the chromosomes have the spindle fibers attached to them at the synaptic points (*XX*). In the arrangements of the group in the metaphase plate, the larger chromosomes, the rings and bent rods, usually lie on the outside and the smaller chromosomes nearer the center. The number in the first spermatocyte, without a single exception, was found to be twelve. Of the group, the three or four extremely large members, the three small ones and the accessory (No. 5) may quite readily be recognized. In polar views of the cell-plate the accessory is very often seen in cross-section or from a very oblique side view. But it can usually be recognized with very little trouble, since it almost always lies near the periphery of the plate and shows no tendency toward division at this time.

Figures 34, 35 and 36 are lateral views, showing the accessory in its characteristic position. All the other chromosomes are in the act of dividing. It is starting ahead of them, undivided, to one of the poles. It may at one time have been in the equatorial plate along with the others, but now, as may be seen by the figures, it is moving out. In a close examination of the spindle it is found that each of the ordinary chromosomes is attached by means of a single fiber to each pole. The fiber, as before said, fixes itself at the point (*X*) on the chromosome where the future reductional division will take place. In the case of the accessory there is only one fiber, that connecting it with the pole towards which it is traveling. Its opposite end is entirely free of fibers.

In figures 37-39, anaphases of the first division, there is clear evidence that the accessory does not divide but passes over, as it is, to one of the resulting daughter-cells. Figure 37 shows all of the remaining chromosomes dividing or divided. The largest one, chromosome No. 12, seems to be much twisted, but nevertheless the four parts to it may be easily made out. The chromosomes in this drawing have been displaced somewhat to right and left in order to show them more clearly. They occupy, however, the same relative position with respect to each other that they did in the cell, although spread out over a greater area. Figures 38 and 39 are still later anaphases, showing eleven dyads at one pole and their eleven mates, plus the accessory, at the other. The halves of the accessory (No. 5, figs. 38 and 39) as it nears the pole seem to be inclined to

fly apart at the distal end, as do the dyads of the other chromosomes. This seems to indicate that the accessory was probably split like the ordinary chromosomes before entering the metaphase. These cells are typical of whole cysts that may be found showing the unequal distribution of the chromosomes to the daughter-cells of the first spermatocytes, thus giving us the two forms of the second spermatocytes.

In polar views of the second spermatocyte metaphase (figs. 40 and 41), half of the cells in a cyst show the twelve and half the eleven dyads.* In these figures a good clew may be obtained as to size relations, for the chromosomes lie spread out flat in the plate, and since all of them are double rods of about the same diameter their relative size is merely a question of length. Of course the size of the accessory here, in comparison with the other chromosomes, is just twice as great as the first spermatocyte, because all the chromosomes here except the accessory are just one-half as large as they were in the preceding mitosis. In figure 41, where there is no accessory, the relative sizes cannot be mistaken. There are the three extremely large members, Nos. 12, 11 and 10, the three small ones, Nos. 1, 2 and 3, and a series 4, 6, 7, 8 and 9 coming between these, many of which are often indistinguishable from each other.

In figures 42 to 45 we have anaphases of the second spermatocyte, showing the division of the dyads in the two kinds of cells. Nos. 42 and 43 are complete cells, showing the division of those that have eleven, and 44, 44*a* and 45 of those that have twelve dyads.* A fragment of the lower halves of each of the latter had to be sought in the next section, but there was no doubt as to the number of chromosomes being twelve. The accessory may be recognized in 44 and 45 by its somewhat greater diameter and more intense staining propensities. In conclusion, it may therefore be said that one-half of the spermatozoa receive eleven chromosomes plus the accessory and the other half eleven and no accessory.

COMPARISON OF SYRBULA ADMIRABILIS WITH SYRBULA ACUTICORNIS.

The first point to be considered is in regard to the number of chromosomes. In this *acuticornis* seems to be different from the whole family *Acrididæ*. So far, with a few exceptions, there has been recorded for the family twenty-three

* The unnumbered elements in figures 41 and 45 are the result of accident in preparing the drawing. They are not present in the cells.

chromosomes in the spermatogonia and twelve in the first spermatocyte. In most cases where exceptions occurred it has been found that they were only apparent, the full number in each case being present, but with some two or three of the members joined together, forming a multiple chromosome. *Stenobothrus,* a form recently studied by McClung, has apparently twenty-one in the spermatogonia and ten or eleven in the first spermatocyte. The other exception is a subfamily of the *Acrididæ,* the *Tettigidæ.* I have now on hand slides of this form showing thirteen chromosomes in the spermatogonia and seven in the first spermatocyte. The variation here need not, however, cause us worry, when we recall how the *Tettigidæ* differ from the rest of the *Acrididæ* in external characters. It seems more reasonable that they should be considered a family by themselves, in rank equal with the *Locustidæ, Gryllidæ* and the *Acrididæ,* than as a subfamily of the last group. The number for the family seems therefore to be constant. Montgomery, however, seems to be in doubt in regard to the number of the chromosomes in *acuticornis.* According to his observations some individuals have twelve, while others of the same species have ten, and in the spermatogonia instead of twenty-three he finds but twenty. Being unable to decide whether the two numbers in the spermatocytes "were due to individual variation or to the presence of two subspecies within the species," he chose to base his conclusions upon the germ-cells of a single individual. It would have been much better to have studied the cells of several individuals and then to have made use of that merely which was typical for the species. In the spermatogonia he "found only two clear polar views of the metaphase, each showing twenty chromosomes." Now the evidence furnished by two cells is not enough upon which to base any important conclusion. There is too much danger of not having the full number present that belong in the cell. Very often a part of the number must be sought in the next section; or sometimes one or two which may have happened to lie on the top or bottom of the section have been dropped out or displaced. He has one other drawing, a late anaphase of the last spermatogonial division, showing the twenty, but at best any late anaphase is a doubtful quantity. In the first spermatocyte he finds ten chromosomes, but he has given not even a single drawing showing this number.

With the mistakes of Montgomery in mind I have been careful in this work to use the testes of several individuals, about a dozen or more, and to examine and draw from these as many cells as time would permit, showing the full number of the chromosomes. The sections were cut twelve micra thick, and so in a great many instances I have been able to show cells that contained in a single section the whole number of chromosomes. In every case so far examined the numbers for the first spermatocyte and the spermatogonia have been respectively twelve and twenty-three. In addition to this the cells of *Syrbula fusca-vittata,* another species of the same genus that was examined, showed the same numbers. In view of these facts *Syrbula acuticornis* either must be radically different in germ-cell structure from the other members of the genus or there must be some mistake in the reported numbers. Until some of the material is inspected I am inclined to believe the latter, for Montgomery in his meager illustrations has not furnished proof sufficient to establish the truth of his statements.

The next point in which we shall have to disagree with Montgomery is in regard to the formation and division of the tetrads. The conditions as they occur in *Syrbula admirabilis* have already been described. There remains to be considered the differing conditions that he has described for *acuticornis,* and concerning which I believe he was mistaken. It seems that his first object here was primarily to find evidence to confirm his old "pre-reduction" theory, and accordingly he has forced the interpretation of every phenomenon, whenever possible, to its support. Generally speaking, it matters little whether reduction occurs in the first or the second maturation division, for the result is the same either way. In fact, some species of the *Acrididæ* (McClung, '05) show that both reduction and equation divisions among ordinary chromosomes may occur at the same time.

As before shown, all the tetrads in the late prophase and in the metaphase may be reduced to a common type—that of a rod with one longitudinal and one transverse division. The latter marks the point at which the proximal ends of the univalent spermatogonial chromosomes have come together to form the bivalent tetrad. This rod has the ability, as before explained, to change its axis from a longitudinal to a transverse direction. The former it possesses in the beginning of synapsis and the latter in the final stages of the first sperma-

tocyte mitosis, just before the separation of the dyads. The cross form is merely a transition stage that the rod passes through in its change from one axis to the other. Montgomery says that "very rarely have the chromosomes an X-shape." By "X" he probably means the cross shape. He is mistaken in this, for the X or cross is a form that may always be seen in any cell during the late prophase and the metaphase. He has but one drawing (his fig. 31) showing a cross form, and for that he gives no explanation whatever. From the way he has drawn it one would judge that the univalent chromosomes which constitute it were in conjunction at the middle of their length instead of at the ends. But this view of the cross cannot be brought into agreement with the form that the earlier more open crosses of the prophase show (figs. 26, 29), where the four parts to the tetrad, the chromatids, may with little difficulty be traced from arm to arm, where it may be seen that in this cross every chromatid forms a part of two arms and no two of the chromatids continue together throughout their whole length (cf. fig. 29).

Before going further we must clear up the confusion about the manner of the attachment of the spindle fibers to the chromosomes. Montgomery's position upon this matter is not clearly defined, but some idea of it may be gotten from the following statement: "All the chromosomes become so placed in the spindle that mantle fibers from one spindle pole are attached to one univalent element, and mantle fibers from the other spindle pole to the other univalent component of each bivalent chromosome." If Montgomery had examined his metaphase chromosomes more extensively and more carefully he would have found many lateral oblique views such as 8 and 11 of figure 32, or in plates XXI and XXII, such as chromosomes 11, 6 and 4 of series 2, or 11 of series 6, 10 of series 10, 7 of series 13, 12 of series 18, 6 and 4 of series 24, or 9 of series 25, all proving beyond a doubt, when carefully studied under the microscope, that the mantle fibers do not attach to univalent halves of bivalent chromosomes. Instead, each fiber is attached to the halves of the two univalent elements that make up the bivalent chromosome, *i. e.*, each fiber (and there are but two fibers running to each ordinary chromosome, one from each pole) carries off or leads off with it to its pole a half of one univalent element together with a half of the other univalent element that constitute the bivalent chromosome. Or

it might be stated in this way: Each fiber does not attach to one of the spermatogonial chromosomes that previously conjugated to form the bivalent chromosome but rather to a half of each member of the pair. The pair the members of which were united end to end becomes a pair split longitudinally, and the result of the splitting is two pairs instead of one pair. Each of the resulting halves or pairs may still be considered a conjugated pair, because it is made up of two distinct spermatogonial elements, as the original conjugated pair was. One of these halves goes to one pole and the other to the opposite pole. The behavior of the chromosomes in a first spermatocyte division, as far as the ordinary chromosomes are concerned, is much like that of those in a spermatogonial division. In both kinds of cells the chromosomes are paired. The difference is that in the spermatocyte the members of a pair during mitosis (as well as at other times) cling together by their proximal ends (sometimes by the distal ends), while in the spermatogonium the members of a pair remain separate. In both cases the division is longitudinal and always begins at the inner or proximal end. The division of the ordinary chromosomes in the first spermatocyte is therefore not a reduction or cross division, not a separation of the members of a spermatogonial pair, but a longitudinal, an equation division, by which each of the resulting daughter-cells receives a half of all the *ordinary* chromosome elements that were present in the mother-cell. The accessory, of course, which does not divide in this mitosis, is an exception and will be taken up later.

Lastly, mention must be made of the chromosomes which Montgomery describes as "irregular V forms" and which he designates by "K." He says that "Whereas ring-shaped chromosomes are frequent in the preceding late prophases they are only very exceptionally found in the equatorial plate, so that probably by the pull of the mantle fibers upon them these ring forms change into the form of the chromosomes lettered *K*." Montgomery has evidently seen no polar views of the first spermatocyte equatorial plate. Figures 30 and 33 of plate XIX and series 1-25 of plates XXI and XXII show without doubt the presence of these ring forms in the metaphase. His chromosome K is merely a lateral or an oblique-lateral view of a ring tetrad that has gone part way into mitosis, and

not an irregular V. If viewed from the pole it would easily be seen to be a ring. The ends or tags pulled out, and to which the spindle fibers are attached, are evidently what he describes as "ends of the univalent halves of the tetrad pulled past each other." In other words, he considers the ring chromosome a loop and these points where the fibers attach, these projections that stick out on opposite sides, the crossed ends of the loop gliding past each other. He believes that one univalent half of the bivalent ring is simply being pulled away from the other half, one spermatogonial member from the other. But I am sure that he is entirely mistaken in this. These tags or projections are the ends (*XX* of the figures) of the transverse axis of the cross (a cross here modified to a ring). He has not taken into account at all the prophase form of the ring (fig. 29), which shows beyond a doubt the origin of these tags or projections. Thére it may be seen that each projection is made up of part of one univalent half and of part of the other univalent half of the ring. The ring may be thought of as a split ring, and the split halves of it as being pulled apart or as starting to move apart in the region (*XX*) where the projections arise, one half going to one pole and the other half to the other pole. This is the explanation that the prophase form of the ring affords, and it would be absurd to argue that the metaphase rings are anything more or less than prophase rings that have become very much condensed. Some of the chromosomes, possibly the smaller ones, may divide transversely, but no evidence has been found to confirm this view. I feel quite sure, therefore, that chromosomes of the ring and cross type do not divide transversely but longitudinally in this first maturation mitosis.

The last and most important correction which I have to make is in regard to the history of the accessory or "heterochromosome." Montgomery describes it as a bivalent element, not univalent, as it has up to this time been described for the *Acrididæ* and for Orthoptera in general. His mistake is due, in part at least, to some large chromatin-like nucleolar structures that are present, at first as two bodies and later as one, in the resting period between the last spermatogonial and the first spermatocyte division. He finds the accessory in the spermatogonia appearing for the first time in the form of a single coiled spireme enclosed within a vesicle of its own, some-

what similar to what Sutton ('00) describes for *Brachystola*. *S. admirabilis* (figs. 7, 8 and 9) agrees with this, but we fail to find that "when the nuclear membrane has completely dissolved away this single loop" (spireme) "segments into two." In his figure 6 it does not seem to me that he has furnished conclusive evidence that his N_2 elements are two heterochromosomes. How can he say for sure that both are heterochromosomes? One may be, but it is a certainty that they both resemble very much some of the other chromosomes. Had he shown the full number of the complex here, the number which he claims for the species, and had proven beyond a doubt that the number was even, not odd, his guess might have had some weight, but he has not shown the whole number in this particular cell nor has he convinced us that the number in the spermatogonia is even. His N_2 pair looks very much like the No. 6's or No. 7's of *admirabilis*. Of course, he claims to find an even number in the metaphase of the spermatogonia, but his two drawings do not furnish sufficient evidence to justify us in accepting this point as proven, especially since it must be accepted in the face of all the other evidence in the family, which in every case happens to be to the contrary. Following out the reasoning, he concludes that each "first spermatocyte receives a half of each of the two heterochromosomes" as well as a half of all the others.

In the reconstitution of the nucleus he again finds that when the "chromosomes begin to elongate and loosen up, two of them do not undergo these changes but remain smooth and dense" (cf. his fig. 13). These he considers identical with his N_2 elements of the spermatogonia. The strange thing, however, is that in his figure 14 the two heterochromosomes should suddenly conjugate and there should appear immediately thereupon, seemingly in the place of one of them, the single nucleolar structure *N*. If these heterochromosomes are to conjugate he should have found previous to his figure 14 three bodies, two heterochromosomes and a nucleolus in each nucleus. According to his figures there were only two bodies at first, the heterochromosomes, and after the conjugation of these there were two bodies still. There seems to be something wrong here, for it is improbable that a nucleolus would appear so suddenly as his element N. It must have been present before. And in addition to this, if his heterochromosomes

conjugated he should have given some intermediate stages showing the two moving together. In some cases (his fig. 20) the N structure is almost as large as his bivalent heterochromosome, which leads us to suspect that probably his nucleolus was in reality what he mistook, part of the time, for one of his heterochromosomes, and that his heterochromosome is not a bivalent structure. In speaking of the bivalence of the latter, at this point, it might be asked why his heterochromosome in the vesicle stage of the prophase of the last spermatogonial division should at first be a single element, then segment and remain separate during the metaphase as two heterochromosomes, and then in the first part of the rest period thereafter between the spermatogonium and the spermatocyte should conjugate again. This, too, seems unreasonable and improbable. From here on, throughout the remainder of the growth period, he finds but two bodies present, the "bivalent heterochromosome" and the "irregular nucleolus."

My work upon *Syrbula admirabilis* gives rise to two views regarding his N_2 structures in his figure 13. The first is that one of the bodies is the accessory seen in end view and the other body, of course, the true nucleolus. But if these structures are exactly the same size, as is quite likely the case, a second and more probable explanation is that he has not seen the accessory at all in this cell but has shown the two nucleoli which we find so often present in *admirabilis* in the early part of the growth period after the last spermatogonial division, as shown in figure 10 of this paper. What adds support to the latter view is the presence of two similar bodies in his figure 1 of a resting spermatogonium. What we have seen in *admirabilis* (fig. 7) convinces me that these two nucleoli in the spermatogonium are not heterochromosomes, for that element may always be found in the reticular condition in a vesicle of its own applied close against the nuclear wall. That there were two of these nucleolar bodies very generally present in *acuticornis* is confirmed by Montgomery in his own words: "With great regularity there is found in each nucleus two or three larger, somewhat irregular, deep-staining bodies. Whether they are nucleoli or heterochromosomes could not be decided by the use of the iron-hæmatoxylin stain." By using Flemming's tricolor stain, and also by careful observation of all prophase stages, I have decided that the bodies in question

are not heterochromosomes, or chromosomes of any kind, but are nucleoli which for a time seem to act like chromatin bodies but which later are found to be plasmosomes or true nucleoli that, on account of their great density, continue to hold the safranin stain for some time. Now after the last spermatogonial division where do these nucleoli go? May it not be possible that they still persist for a while as in figure 10? It seems quite probable that the nucleoli (what Montgomery terms heterochromosomes) of his figure 13 are the same as those of my figure 10, and that his heterochromosome as it lies in the region of the periphery of the nucleus may have been overlooked, for after the last spermatogonial division it is often difficult to distinguish it from the ordinary chromosomes, since they all seem to be in a more or less semifluid and also semireticular condition, much like the accessory itself.

In Montgomery's figures following (15, 16, 17, 18, 19, 20) his accessory is shown in its characteristic position near the nuclear wall. He is probably correct in thinking that it shows longitudinal splitting but wrong in maintaining that it shows transverse constriction. In *admirabilis* it has shown at no time a transverse constriction, nor was the longitudinal splitting noticed until the anaphase of the first spermatocyte division. That, however, does not prove that the splitting may not be present in the growth period. The bent accessories of his figures 18, 20, 24, 26 and 30 are not evidence of the bivalency of this element, for it often bends or coils upon itself, as shown by my figures 23-28, and the bend may occur in any region, not in the middle alone, and seems to be due to the extreme length. During its spireme stage it passes through a variety of shapes. At first it is much convoluted. Then as it shortens and thickens it usually lies bent upon itself for a time, but finally it becomes an almost straight rod.

Montgomery's Y chromosome of figure 33, which was tardy in coming to the equator but which he claimed did so later on, although he gave us no assurance of it in his drawings, is in all probability the accessory chromosome, for it agrees with what we have found for *admirabilis*, where in every cell at this stage it may be seen starting off undivided ahead of its fellows to one of the poles. Montgomery was wrong, however, in supposing that it had not yet taken up its position in the equator. In fact, it has already been there, as the dividing of

the other chromosomes indicates, and is now on its way to the lower spindle pole. That this is the case is shown also by the spindle fibers, for they are evidently exerting an influence on the ordinary chromosomes, since the latter are in the act of dividing. The accessory, as his own drawing shows, is attached by these fibers to but one of the poles, and that fiber must evidently be pulling it toward its pole. This is a point in which *acuticornis* agrees entirely with *admirabilis* (figs. 34, 35, 36). But chromosome Y of his figures 32 and 34 is positively not the accessory, because that element, we have found, does not divide in the first maturation mitosis but passes over undivided (figs. 37, 38 and 39) to one of the daughter-cells or second spermatocytes. He was therefore wrong in identifying it with the Y of his figure 33. If he had examined the full number of chromosomes in these cells (his figs. 32 and 34) he would undoubtedly have found the accessory in its peculiar position starting off ahead of its fellows, as he found it in figure 33.

The heterochromosome of *Syrbula* is therefore not a bivalent structure, for it is paired at no time during its whole course. It does not divide in the first maturation mitosis but in the second. Montgomery quotes McClung as describing "for *Hippiscus* an accessory chromosome of the spermatocyte, said to divide in both maturation mitoses." This is a mistake. McClung gives no such description. As he was unable at the time to follow it farther or to distinguish it from the ordinary chromosomes after it had taken up its position in the metaphase of the first division he merely supposed that it must behave like the other chromosomes, and with that remark left it, expressing the intention of making it the subject of a subsequent paper. Since that time it has been found to behave as in other Orthoptera, dividing only in the second division.

And now Montgomery attempts to reconcile the "double heterochromosomes" of the Hemiptera with the single heterochromosome or accessory of the Orthoptera. His suggestion is as follows: "In the Orthoptera the heterochromosome is single in the spermatogonia; single therefore in the spermatocytes, it does not divide in the first maturation mitosis, but does in the second. Because it does not divide in the first mitosis it must be either univalent or else, already in the spermatogonia, be composed of two so firmly united that they can-

not be divided in the reduction mitosis." In reply to this the results obtained on *admirabilis* have shown beyond a doubt that the accessory is not paired in the spermatogonia. In addition to this it might be said that no workers upon the Orthoptera, except Montgomery himself, have ever found this element to be a bivalent structure, and more than this, it has been shown by Wilson ('05) that in the majority of the Hemiptera even, where Montgomery found most of his evidence for a double heterochromosome, the accessory (heterotropic chromosome) is unpaired, and Wilson believes that "it will be found to be so in all" Hemiptera. Montgomery's attempt, therefore, to bring these two classes into harmony, was first of all based upon inaccurate observations, and since that time has accordingly been rendered entirely unnecessary by more thorough researches.

His final summary is that "all chromosomes and heterochromosomes, be they paired or single in the spermatogonia, divide reductionally in the first maturation mitosis, whether the division consists in two univalent components separating from each other or a single component passing undivided into one of the second spermatocytes." As far as the Orthoptera are concerned it is true that in the case of the single component passing undivided into one of the daughter-cells there is a reduction, for only one-half of the second spermatocytes receive thereby this chromosome, but it is not true that all the other chromosomes in the complex divide reductionally in this mitosis, proof of which has been amply afforded by the behavior of the rings and crosses in *Syrbula admirabilis*, in *Hippiscus* (McClung, '00), and in the *Locustidæ* (McClung, '02).

INDIVIDUALITY AMONG CHROMOSOMES.

The most live problems to-day in the field of cytology are those which center around the belief that chromosomes are discrete individual elements, which in every nucleus are constant and definite morphological structures, occurring from generation to generation and bearing in themselves the power to transmit the characters of the species. Therefore everything that in any way adds to our knowledge of this subject, either new or in confirmation of what has already been learned, will be of interest. The chromosomes of *Syrbula admirabilis* are well adapted for a study of these questions on account of their favorable separation from each other in the metaphase figures,

their unusually large size, and especially on account of the great variation in size among the members of the complex. The tables which have been arranged in plates XXI and XXII were gotten up from such complexes as are represented by figures 30-33, 40, 41 and 1-6. In these tables the twelve chromosomes of a cell are strung out in a horizontal row or series. Each individual chromosome is given a position in its series according to its size, the largest, No. 12, coming first, No. 11 second, etc. The series are placed one below the other, so that like chromosomes will appear in the same column. In this way all the No. 12's throughout the series of cells will be found in the first column, the No. 11's in the next, and so on for all the chromosomes belonging to each specific size or rank. In the plates the first twenty-five series are from that many polar views of first spermatocyte metaphases and were taken from four different animals (A, B, C, D). In series 24 and 25, under D, the chromosomes seem much larger than in any of those preceding. This was due to the stain used, Flemming's tricolor, which has a tendency to swell the structures. The chromosomes of the other series were stained with iron-hæmatoxylin. In series 26-29 are shown the chromosomes of the two forms of second spermatocytes, those with the accessory and those without, and in 30 and 31 two series from spermatogonial metaphases (taken from figs. 2 and 6, plate XVIII).

In arranging the members of a series it was often difficult to determine the relative position of some of those nearest in size, because in some cases there was very little size variation while in others the true size was hidden on account of the position of the chromosome in the cell-plate. The latter was the case when ring-like forms were turned on edge or when those of a rod or kidney shape were viewed from the end. But although there may be doubt in regard to the position and size of those nearest each other in the series, such as Nos. 6 and 7 or 8 and 9, there is absolutely no danger of confusing those of such wide range as Nos. 12 and 6, or 10 and 5, or even of those as near as Nos. 10 and 7.

There are in a general way six columns (12, 11, 10, 9, 7, and 4) in which ring-like forms occur. The other columns contain chiefly straight or bent rods. In regard to the ring-like forms a word of explanation is here necessary. A ring, as has

been explained by others and once already in this paper, consists of two homologous spermatogonial chromosomes joined together by their ends, proximal end with proximal and distal with distal. The proximal ends are always united, but the distal ends may or may not be. Sometimes they are so firmly joined that the distal end or region of the ring cannot be told from the proximal except by the fact that the proximal portion always points towards or lies nearest to the center of the cell-plate. In these tables (plates XXI and XXII) the rings are always placed as nearly as possible in a position with the proximal portion uppermost. In the case of the kidney-shaped or bent-rod forms in columns 8, 6, 4 and 3, and any similar chromosomes that may occur in other columns, the proximal end or part (the convex side) of the chromosome is turned towards the left. To be logical this portion in these chromosomes should have been turned upwards, as in the ring forms, but less room was required the other way. In the case of the accessory the proximal end is again as far as possible placed uppermost. In series 26-31, from the spermatocytes and the spermatogonia, the proximal end, that to which the fibers are attached, is always placed uppermost.

In the formation of the rings quite often the distal ends are imperfectly or not at all united, but the polar or proximal ends are always united. It does not seem to be necessary that the distal ends shall always unite, but there is a tendency for them to do so, and this tendency is greater in certain individual chromosomes than in others. In general, the larger chromosomes seem to be more inclined to form rings than the smaller. Nos. 12 and 11, according to these cells, are always rings or ring-like forms. Some possible exceptions occur, principally in the second column (chromosome 11 in series 2, 8, 19, 22 and 23) where the distal ends are either imperfectly or not at all united. But nevertheless the distal ends of the pair bend around toward each other, showing that they have a tendency to unite and form a ring. In series 5 and 6 of column 10 two notable exceptions occur: that in series 5 is somewhat inclined to form a ring, but that in 6 has no inclination whatever to do so. In series 18 the distal ends of No. 10 seem to have glided past each other. In such as that in 15, 16, 20, 21 and 23 the distal ends have not moved past each other, but they evidently did not meet exactly on end, as is shown by the

drawings. Some chromosomes, such as 12 and 11 of series 2, or 10 of series 19, show by the length of their transverse axis that they have advanced farther in mitosis than many of their neighbors. The chromosomes in column 9 seem all to belong to the ring-like form. That in series 6 is not a ring now but it may have been before it became drawn out so far. This is a good example of a chromosome completely elongated in the transverse direction. The No. 8's are all very constantly kidney or bent-rod forms. There is very little difference in size between them and the No. 7's. Of the No. 7's, nineteen out of the twenty-five are rings. The others are usually of kidney form. That in series 20 is rod-shaped. The point at which the fibers attach is indicated by the *X*. In column 6 the chromosomes are all kidney-shaped with two (series 23, 25), possibly one, exceptions. The exceptions may be considered ring forms, but they may also be bent rods, for one of them (series 23) has the distal ends of the two halves of the ring not united at all, while the other (6 of 25) is so nearly the size of chromosome 4 of the same series, which is not a ring, that possibly I may have the two interchanged. In column 5 is the accessory. There is no trouble in finding the proper place for this element, since it may always be recognized in the cell by its form, and especially by its position in the cell-plate (figs. 30-36.) In size it seems to fit between the No. 4's and 6's. On account of its habit of starting off to the pole ahead of the other chromosomes it so happens that it nearly always appears either in cross-section or a very much foreshortened side view. Sometimes, but not often, it is seen in full lateral view in the metaphase plate. As to individuality there is no member of the complex that is more pronounced than the accessory. It is the only element that may be traced from the prophases of the spermatogonia through all the stages of the growth period and the division stages of the first and second spermatocytes up to the spermatids. It seems at times to be an element peculiar unto itself, and yet when viewed in the metaphases it behaves very much like the other chromosomes, even so much so that it is with difficulty distinguished from some of them (the 4's and 6's in first spermatocytes and 10's in spermatogonia). In all respects it is a chromosome, but one whose individuality as to form, size, and habits is very strongly marked. In column 4 about one-half the number are rings

and one-half are kidney-shaped. The small chromosomes (3's, 2's and 1's) are always rods. The largest of them may show a kidney shape. Sometimes in far advanced metaphases, as in series 1 and 2, the second smallest (No. 2) is divided.

A study of the chromosomes as they appear in these series furnishes very strong evidence that they are discrete individual elements. There are three means by which chromosomes may show their individuality—by size, by shape, and by function. Boveri ('02) proved for them an individuality of function, Sutton ('02) that of size, and Baumgartner ('04) that of shape or form. The series here arranged suggest nothing in regard to function, but they do point to an individuality of size and to a certain extent of form. Of the two latter characters size seems to be of most consequence. The form of a chromosome seems not to be essential, for it may vary to a certain extent, but the size remains the same. A very clear idea of this size relation may be obtained by observing the second spermatocyte series, 26-29, for in any one of these series all of the members except the accessory are of comparatively uniform diameter, and to get the size relation all we need to do is to compare their lengths. In the spermatogonial series (30, 31) exactly the same size relations may again be seen. This size relation is a constant. In the first spermatocytes every cell shows a series, or complex, of chromosomes, every member of which bears to its fellows the same relation in size as do those of any other cell. If any of the group in the cell are large they are all proportionately large; if small, they are all likewise small. The relative sizes remain the same in either case. Then, too, this size relation is the same from one generation of cells to the next as well as among different cells of the same generation. For the proof of this compare the series from the spermatogonia and second spermatocytes with those of the first spermatocytes.

In cells from each of these generations it has been observed that there are always two groups of chromosomes in the complex that may easily be recognized, the three extremely large ones (12, 11, 10) and the three extremely small (3, 2, 1). They may be seen in the prophase stages as well as in the metaphase. The grouping seems to be characteristic of the *Acrididæ*, for it is found in all three of the subfamilies. Sutton ('02) described it for *Brachystola*, one of the *Œdipodinæ*,

Nowlin ('08, just being published) for *Melanoplus,* one of the *Acridiinæ,* and Montgomery ('05) *Syrbula acuticornis,* one of the *Truxalinæ.* Whether there is anything of particular significance in the fact or not will of course appear later on. The presence, however, of this grouping in every cell is again evidence of the individuality of the chromosomes, for it is a certainty that the members of these two groups are entirely distinct and different individuals. There is no danger of ever confusing any such large chromosomes as Nos. 12, 11 or 10 with any so small as Nos. 3, 2 or 1. The same might be said of any of the intermediate members of the series, those which approach each other more nearly in size, were we able to distinguish between them by their sizes. But the proof afforded by the extreme members of the complex is enough to establish individuality for all.

Shape or form among chromosomes, as mentioned above, does not seem to be a matter of first importance but is dependent in a measure upon size. It is true that some chromosomes, as Nos. 12 and 11 or 9, were found to be always rings throughout the twenty-five cells that were studied, but again No. 10 was found to have two out of the twenty-five that were not rings, and possibly if we had searched far enough the 12's, 11's and 9's would have shown similar variations. Yet the two No. 10's that did not form rings were in all probability as truly No. 10 chromosomes as any other No. 10's in the column, for No. 10 in series 5 or 6 bears the same size relation to its fellows in the series as do any of the ring-form No. 10's to their fellows. It is not essential that No. 10 be a ring, but it is essential that the limbs of 10 be long and that it contain the same amount and the same kind of material that all other No. 10 chromosomes contain. The fact that the limbs of this chromosome are long and slender makes it more possible for it to form a ring, since the limbs have a better chance to bend around toward each other. This possibly might in a general way account for the fact that more rings are found among large chromosomes than among small. The latter, however, is not always true, for chromosome No. 4, which is very small, may often be a ring, while No. 8, a much larger chromosome, is never a ring. Possibly the thickness and corresponding shortness of the limbs of the No. 8's may be the reason why they never form rings. Nos. 7 and 4

seem to be indifferent as to whether rings or kidney shapes are formed, but their limbs are usually slender and show that they may bend around to each other easily. The extremely small chromosomes, Nos. 1, 2 and 3, of course cannot form rings, on account of their shortness. Assuming that the diameter of the rods or univalent arms forming the chromosome is approximately equal for most of the chromosomes, the large chromosomes would then have a tendency to form rings because their limbs are long; the small one to form rods, and especially straight rods, because their limbs are short; and those that come between to form bent rods, V's and rings indifferently because their limbs are either too short always to insure rings or too long always to form rods. The question then as to whether a chromosome shall be a ring or not may be considered as depending in a general way upon the relation existing between the length of its limbs or arms and their diameter. Assuming, as we did, that this diameter tends to remain a constant, the chief determining condition would then be size, and we know the size relation in a chromosome is a constant, therefore the form which is dependent upon size would tend to be a constant. But it is not essential that these chromosomes shall always form rings or not, for the truth of the matter is that they are not always constant in this respect. Undoubtedly other agencies enter in to modify this secondary character, such as condition of the cytoplasm, position of the cell in the cyst, the degree to which the gliding of the chromatids had advanced in the tetrad before the condensation process of the metaphase had overtaken it. In respect to the cross form it may here be said that the fact that a chromosome appears as such is of no special importance in individuality, for, as has been already shown in this paper, all the ordinary chromosomes pass through this condition. It too may be considered of secondary importance. Many of these agencies thus working together no doubt prevent the chromosomes from doing exactly as they would otherwise do; and if such be the case it would only be that much more proof that the form of a chromosome is not a first essential, not an entirely reliable character upon which to judge individuality. These external conditions, however, do not alter the size relations of the chromosomes. That remains a constant under all circumstances. It seems fair to conclude, therefore, that form, though it ought

to receive due consideration, is not to be held as a first essential in the determination of individuality in the chromosomes but rather as a secondary character dependent to a large extent upon size.

SUMMARY.

1. There are twenty-three chromosomes in the spermatogonia. They may be arranged in a graduated series of eleven pairs plus one odd unpaired member, which is most likely the accessory. In this series there may readly be recognized three extremely large pairs and three extremely small pairs. The members of the pairs do not always lie together in the equatorial plate.

2. The accessory chromosome is seen for the first time in the prophase of the spermatogonia, where it may be recognized by the peculiar vesicle within which it lies, located at the periphery of the nucleus. It is at this time reticular in structure, like the other chromosomes. After the last spermatogonial division it remains condensed, while the ordinary chromosomes become reticular and diffuse. As usual in *Acrididæ* it lies in close contact with the nuclear wall, and it continues to maintain this peripheral position even in the spindles of the two maturation divisions that follow. In the growth period it passes from the telophase condition of the last spermatogonial division to a lump condition. Out of this lump is evolved later a long, slender, much-coiled spireme. This spireme continues to shorten and thicken, and the chromosome finally enters the metaphase as a short, thick, almost straight rod. During all this time this chromosome has shown no signs of being a paired or bivalent structure. In the prophase of the spermatogonia there was no spireme segmented into two parts. In the spermatogonial metaphase it was not paired, for the spermatogonial number of chromosomes was odd, not even. In the telophase of the last spermatogonial division, and in the resting and growth period following, it was not paired, for the second dense chromatin-like body present there was found to be a nucleolus, not a heterochromosome. In the latter part of the growth period and in the prophase of the first spermatocyte, when the chromosome had assumed the spireme condition, no constriction or bending at any particular place (the middle) was met to indicate that the element had already been paired before leaving the spermatogonia. On the con-

trary, it was found to be, under all conditions and at all times, a univalent chromosome.

3. In the prophases of the spermatogonia two deeply staining nucleoli are usually present. In the telophase of the last division and in the early part of the growth period following they are still present, staining red with safranin, like the accessory. In the middle and later growth period there is but one large nucleolus present, almost as large as the accessory. It fades out and disappears as the chromosomes enter the late prophase of the first spermatocyte and begin to condense.

4. There are twelve chromosomes in the first spermatocyte. No exceptions to this were found in a series of fifty or more cells that were examined, belonging to at least a dozen different animals. The chromosomes consist of rings, semiclosed rings, crosses, bent rods and straight rods. All of these forms may be considered as modifications of a single type, that of a rod with one longitudinal and one cross division. The cross division locates the point at which the spermatogonial pair conjugated by their proximal ends. This rod has the ability to change its long axis from a longitudinal to a transverse direction. All chromosomes of the first spermatocyte, except the accessory, pass through this process of change of axis. The cross form represents merely a transition stage in the process. Rings are merely spermatogonial pairs that have conjugated at both proximal and distal ends. Semiclosed rings, kidney shapes, bent rods and straight rods are those that have conjugated by the proximal end only. All the ordinary chromosomes divide in the first division. They divide longitudinally (equationally) not reductionally. The accessory does not divide but passes over complete to one of the resulting daughter-cells.

5. One half of the second spermatocytes therefore contains eleven chromosomes and the other half eleven plus the accessory. These dyads here correspond to pairs of homologous spermatogonial chromosomes. They are divided crosswise or reductionally. It is here that the maternal and paternal elements are separated from each other. The accessory divides longitudinally here like it did in the spermatogonia. One half the spermatids receive eleven chromatids and the other half twelve. This of course insures a dimorphism of the protozoa.

6. The chromosomes show individuality of size and, to a

certain extent, of form, but form is more or less dependent upon size. Size is a constant relation among the chromosomes, therefore form, which is dependent upon size, would have a tendency to be a constant. But it is not always a constant, *i. e.*, a chromosome which is usually a ring is not always a ring, although it tends to be. This is probably due to external conditions. Since this variation is encountered, form cannot be essential; hence not an entirely reliable character upon which to base individuality.

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